

Epi proColon®

A Blood Test for Colorectal Cancer Screening

Real-Time PCR • Single-Day Test Protocol • Flexible Workflow

What is the Epi proColon Blood Test?

Epi proColon is a blood test that detects methylated Septin9 DNA, a differential blood biomarker found to be specifically hypermethylated in colorectal cancer, and not normal colon tissue. Hypermethylated Septin9 tumor DNA shed into the bloodstream displays a unique and discriminating methylation pattern that is detectable in plasma by Real-Time PCR.^{1,2}

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The Epi proColon Test Intended Use

The Epi proColon test is a qualitative *in vitro* diagnostic test for the detection of methylated Septin9 DNA in EDTA plasma derived from patient whole blood specimens. Methylation of the target DNA sequence in the promoter region of the *SEPT9_v2* transcript has been associated with the occurrence of colorectal cancer (CRC)¹. The test uses a real-time polymerase chain reaction (PCR) with a fluorescent hydrolysis probe for the methylation specific detection of the Septin9 DNA target.

The test is indicated to screen patients for colorectal cancer who are defined as average risk for colorectal cancer (CRC) by current CRC screening guidelines. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy. Men and women 50 to 85 years of age were included in the Epi proColon clinical trial. The Epi proColon test results, together with the physician's assessment of history, other risk factors, and professional guidelines, may be used to guide patient management.

The Epi proColon test is for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument.

Warnings

- The Epi proColon test is not intended to replace colorectal screening by colonoscopy.
- The Epi proColon test is not intended to screen persons under the age 50 who are considered to be at average risk for colorectal cancer.
- Positive Epi proColon test results are not confirmatory evidence for the presence of colorectal cancer. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy.
- A negative Epi proColon test result does not guarantee absence of cancer. Patients with a negative Epi proColon test result should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.
- Positive test results have been observed in clinically diagnosed patients with chronic gastritis, lung cancer and in pregnant women.³

Note: The Epi proColon test has not been tested in persons considered to be at higher-risk for colorectal cancer.

Detecting Methylated Septin9 DNA

Cytosine residues in the v2 region of the *SEPT9* gene become hypermethylated in colorectal cancer tissues. When plasma samples are treated with a high concentration of bisulfite, unmethylated cytosines are converted to uracil while methylated cytosines remain unchanged, see Figure 1. As a consequence of treatment, the DNA sequence is altered based on methylation status and can be analyzed by PCR amplification, see Figure 1.^{1,2}

HeavyMethyl® Core Technology

The Epigenomics' HeavyMethyl core technology combines the use of primers that amplify the target biomarker regardless of methylation status, with a blocking oligonucleotide to suppress the amplification of unmethylated DNA, and a methylation-specific probe to detect the amplified methylated sequence, see Figure 2. The proprietary HeavyMethyl core technology enables detection of low copy number tumor DNA in a background of non-tumor DNA in plasma.^{1,2}

Figure 1: Detecting DNA Methylation

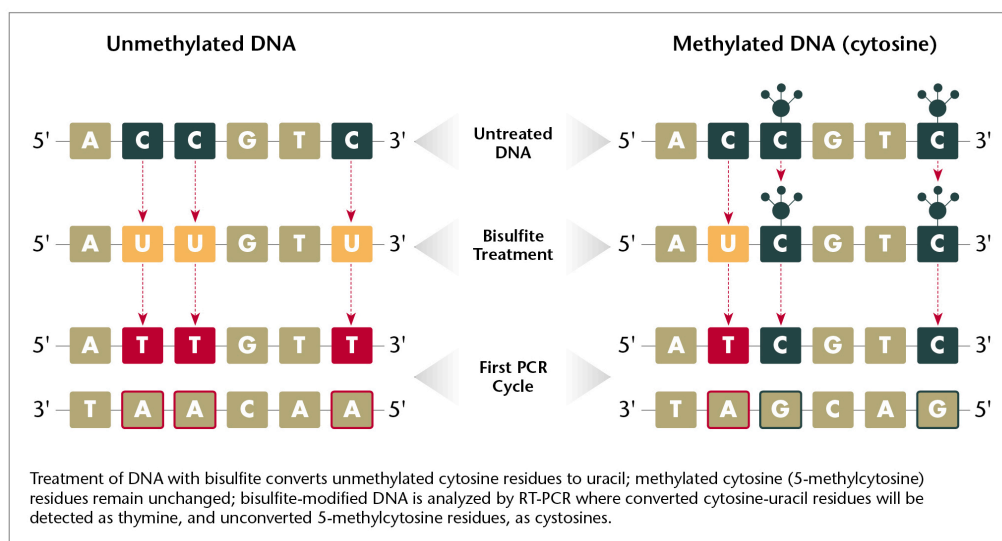
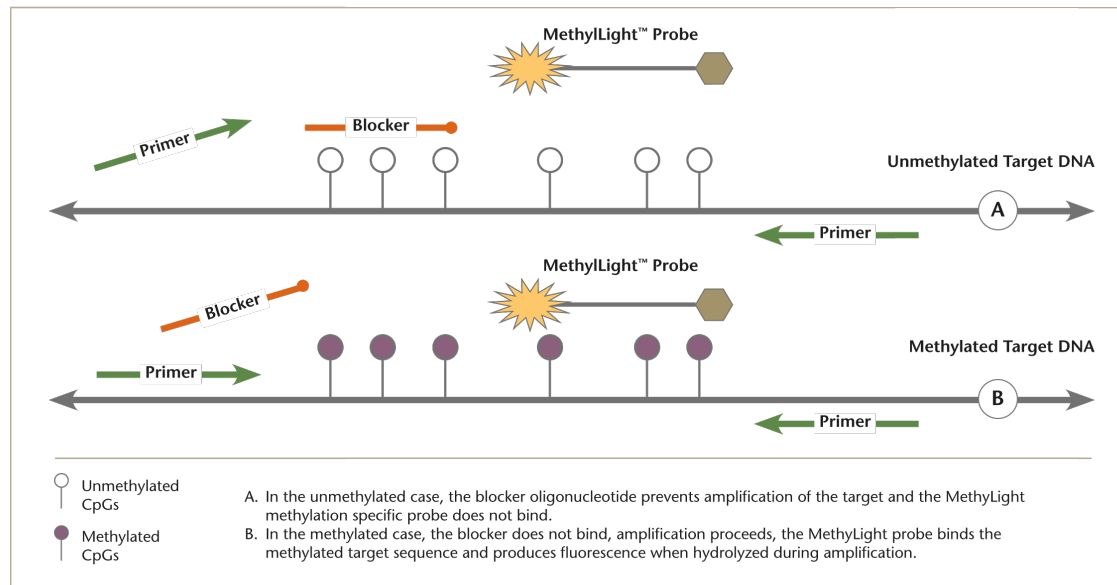


Figure 2: HeavyMethyl® Real-Time PCR

FIGURE 3: HeavyMethyl® Real-Time PCR



FEATURES AND BENEFITS

COMPLETE TEST KIT OFFERS CONVENIENCE & EFFICIENCY

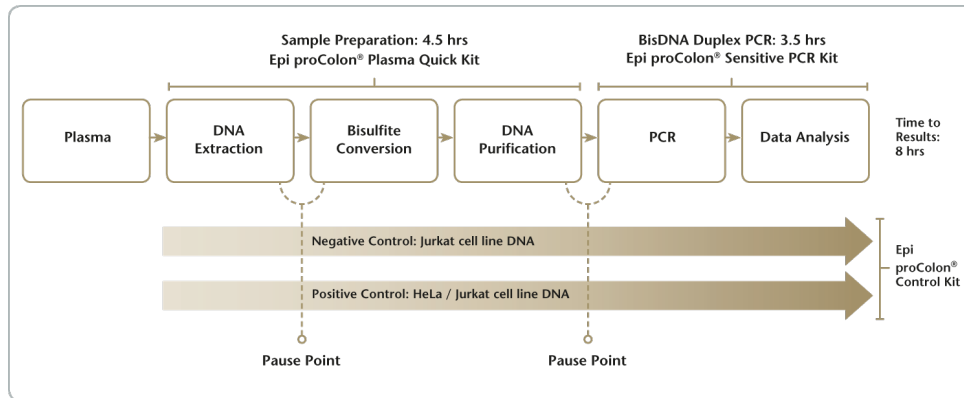
- DNA Extraction Reagents
- PCR Reagents
- External Positive and Negative Controls

QUALITY CONTROL VERIFIES WORKFLOW & VALIDITY

- **Internal Process Control:** Co-amplified internal control monitors sample quality, sample preparation and adequate DNA concentration
- **External Controls:** Positive and Negative Controls performed identically to patient samples monitor successful workflow and ensure validity of patient test results

SIMPLE REAL-TIME PCR TEST — BASIC MOLECULAR LAB TECHNOLOGY

- Familiar PCR technology
- Flexible workflow adapts to staff workload requirements
- Single day protocol with TTR usually < 8 hours

Figure 3: The Epi proColon Test and Workflow (use some version of this)

1. Plasma Sample
2. DNA Isolation
3. Bisulfite Conversion and Purification
4. Duplex HeavyMethyl Real-Time PCR
5. Data Analysis

Clinical Summary

In a large, prospective multicenter clinical trial, 7,941 women and men ages 50 to 85, who were of average-risk for colorectal cancer were enrolled at 32 clinical sites in the US and Germany.³ The Epi proColon test's clinical performance has been validated in 1,544 of the trial participants using colonoscopy as the reference standard. The study included all patients with cancer (all stages) or advanced adenomas and a subset of patients with small polyps, and patients with no evidence of disease (NED), Tables 1 and 2.

Table 1: Epi proColon Clinical Trial Results for Different Patient Groups: Specificity = 70% (1182/1500)

Classification	Positives (Total)
NED (No Evidence of Disease)	97 (444)
Polyps	87 (435)
Advanced Adenomas	134 (621)

Table 2: Epi proColon Clinical Trial Results for Colorectal Cancer (CRC) Stages: Sensitivity = 68% (30/44)

CRC Stage	Positives (Total)	Percent Positive
Stage I	7 (17)	41%
Stage II	10 (12)	83%
Stage III	8 (10)	80%
Stage IV	5 (5)	100%
Total Cancers	30 (44)	68%

In a second, large multicenter clinical trial, the Epi proColon test was compared to a widely-used US commercial fecal immunochemical test (FIT). The study was designed to collect matched blood and fecal specimens and clinical data from screening guideline-eligible subjects using colonoscopy as the reference method for detection of CRC. Subjects were recruited at 61 clinical sites in the US according to the following scheme:

- Subjects having CRC or a high suspicion of invasive CRC identified during screening colonoscopy were enrolled and provided blood and fecal samples at least 10 days after colonoscopy but prior to surgery or intervention
- Prospectively enrolled subjects provided blood and fecal samples prior to bowel prep for screening colonoscopy

Of 337 subjects enrolled in the study, 36 were excluded due to failure to meet inclusion/exclusion criteria. From the remaining 301 enrolled subjects, there were 101 patients with colorectal cancer (CRC), 29 with advanced adenomas (AA), 77 with small polyps (SP) and 94 with no evidence of disease (NED). Plasma samples were available from all 301 subjects. Fecal samples were not available from 11 subjects (4 CRC, 2 AA, 2 SP and 3 NED).

Results from the Epi proColon and FIT tests were compared to results obtained with colonoscopy, as shown in Table 3. Tables 4 - 6 present sensitivity and specificity results for the Epi proColon test and the FIT test.

Table 3. Three – way comparison of Epi proColon, FIT and Colonoscopy results.

Diagnostic Accuracy Criteria: Standard Colonoscopy						
Colorectal Cancer				Non-Colorectal Cancer AA, SP, NED		
	Epi proColon Positive	Epi proColon Negative	Total	Epi proColon Positive	Epi proColon Negative	Total
FIT Positive	50	16	66	1	4	5
FIT Negative	20	11	31	36	152	188
Total	70	27	97	37	156	193

Table 4: Epi proColon Sensitivity and Specificity for all samples (n=301)

Epi proColon Test		95% CI	
Sensitivity	73.3% (74/101)	63.9%	80.9%
Specificity	81.5% (163/200)	75.5%	86.3%

Table 5: Epi proColon Sensitivity and Specificity for paired samples (n=290)

Epi proColon Test		95% CI	
Sensitivity	72.2% (70/97)	62.5%	80.1%
Specificity	80.8% (156/193)	74.7%	85.8%

Table 6: OC FIT-CHECK Sensitivity and Specificity for paired samples (n=290)

FIT Test		95% CI	
Sensitivity	68.0% (66/97)	58.2%	76.5%
Specificity	97.4% (188/193)	94.1%	98.9%

The observed sensitivity for CRC on paired samples was 4.2% higher for the Epi proColon test (Table 5 & 6). The sensitivity of the Epi proColon test is statistically non-inferior to the FIT test.

For specificity, the difference between tests was 16.6% in favor of the FIT (Table 5 & 6). This result does not demonstrate non-inferiority for specificity.

Results Interpretation

- A **POSITIVE BLOOD TEST RESULT** indicates that methylated Septin9 DNA has been detected in the plasma sample tested. Methylated Septin9 DNA has been associated with the occurrence of colorectal cancer.¹ Because the Epi proColon test is not a confirmatory test for the presence of colorectal cancer, patients with positive Epi proColon test results should be referred for diagnostic colonoscopy.

Note: Positive test results have been observed in clinical studies in patients with chronic gastritis, lung cancer and in pregnant women.^{3,4} Because a colonoscopy procedure examines the entire anterior lining of the colon and rectum, CRC is unlikely when no abnormal findings are discovered during this procedure.
- A **NEGATIVE BLOOD TEST RESULT** indicates the absence of methylated Septin9 DNA in the plasma sample tested. Because a negative test result is not confirmatory for the absence of colorectal cancer, persons should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.

Note: Studies show that methylated Septin9 DNA is not present in plasma from all patients with colorectal cancer and therefore, a negative test result does not guarantee absence of cancer.³ Detection of CRC is dependent on the amount of circulating tumor DNA in the plasma specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage.³

The Epi proColon Test Kit

30 Patient Plasma Samples • 2 Controls • 96 Well Format

PROVIDED	REQUIRED
<ul style="list-style-type: none"> Epi proColon Plasma Quick Kit (M5-02-001) Epi proColon PCR Kit (M5-02-002) Epi proColon Control Kit (M5-02-003) 	<ul style="list-style-type: none"> BD Vacutainer® K2 EDTA Blood Collection Tubes[†]

REQUIRED INSTRUMENTATION

Life Technologies™ Instrument and Software Specification^{††}

- Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument, with
- SDS v1.4 21, CFR Part 11 Module

[†]This product has been validated ONLY for use with these EDTA collection tubes; Other reagents and consumables as required for Real-Time PCR are detailed in the Epi proColon Test Kit Instructions for Use (IFU)³.

^{††}This product has been validated ONLY for use with the Applied Biosystems 7500 Fast Dx Real-Time PCR instrument and software system.

Refer to the Epi proColon Test IFU for more information regarding user requirements³

CPT Code Information

81401, Molecular pathology procedure, Level 2, *SEPT9* (Septin9)(eg, colon cancer), methylation analysis.

Precautions, Contraindications and Warnings

Precautions

- The Epi proColon test is an alternative screening method for patients who are defined as average-risk for colorectal cancer by current screening guidelines.
- The Epi proColon test was positive 2 out of 10 times when colorectal cancer was not present. The Epi proColon test was negative 3 out of 10 times when colorectal cancer was present.
- Detection of colorectal cancer is dependent on the amount of free circulating tumor DNA in the specimen and may be affected by sample collection methods, sample storage, patient factors and tumor stage. Some CRC tumors may not shed methylated Septin9 into the blood.
- There is insufficient evidence to report programmatic screening sensitivity for the Epi proColon test over an established period of time.
- CRC guideline recommendations vary for persons over the age of 75. The decision to screen patients over the age of 75 should be made on an individualized basis through shared decision-making with your patient.
- The Epi proColon test demonstrated non-inferiority to a FIT test (OC FIT-CHEK® Polymedco), for sensitivity but not for specificity, indicating that the Epi proColon test exhibited a higher rate of false positive results compared to the FIT test.
- The Epi proColon test has been validated for use only with plasma derived from blood collected with BD Vacutainer® blood collection tubes (Becton Dickinson). Do not use this test with other clinical specimen types or with other blood collection tubes.
- As with all screening methods, test results should be interpreted by a healthcare professional.

Contraindications

- The Epi proColon test was not tested in persons considered to be at higher-risk for developing CRC. Persons at higher-risk for developing CRC include those with a family history of CRC, particularly two or more first degree relatives with CRC or one or more first degree relative(s) less than 50 years of age with CRC, personal or family history of benign polyps in the colon or rectum, Crohn's disease, inflammatory bowel disease, genetic syndromes like Lynch syndrome (hereditary non-polyposis colorectal cancer) or FAP (familial adenomatous polyposis) and other lifestyle factors.
- This test was also not evaluated in persons with anorectal bleeding or hematochezia or with known iron deficiency anemia.
- For persons considered to be at higher risk for colorectal cancer, a screening and management plan that also includes colonoscopy should be considered.⁵
- For patients diagnosed and treated for colorectal cancer, a disease monitoring and management plan should be discussed with the patient based on their individual health history.⁶
- If a person has signs and symptoms of colorectal cancer, a diagnostic colonoscopy is recommended.⁵

Warnings

- The Epi proColon test is not intended to replace colorectal screening by colonoscopy.
- The Epi proColon test is not intended to screen persons under the age 50 who are considered to be at average risk for colorectal cancer.
- Positive Epi proColon test results are not confirmatory evidence for the presence of colorectal cancer. Patients with a positive Epi proColon test result should be referred for diagnostic colonoscopy.
- A negative Epi proColon test result does not guarantee absence of cancer. Patients with a negative Epi proColon test result should be advised to continue participating in a colorectal cancer screening program that also includes colonoscopy, fecal tests and/or other recommended screening methods.
- Positive test results have been observed in clinically diagnosed patients with chronic gastritis, lung cancer and in pregnant women.^{3,4}

References and Resources

1. deVos T et al. Circulating methylated *SEPT9* DNA in plasma is a biomarker for colorectal cancer. Clin Chem. 2009, (55):1337-1346.
2. Lofton-Day C et al. DNA methylation biomarkers for blood-based colorectal cancer screening. Clin Chem. 2008, (54)2:414-423.
3. Epi proColon Instructions for Use (IFU) and Epigenomics data on file.
4. Warren, D. et al. Septin 9 methylated DNA is a sensitive and specific blood test for colorectal cancer. BMC Med. 2011, (9)133.
5. American Cancer Society. Colorectal Cancer Facts and Figures, 2011-2013.
6. Screening for Colorectal Cancer. Clinical Summary of the United States Preventive Services Task Force (USPSTF) Recommendation, 2013.

	Web Address
CRC Resources	
American Cancer Society	www.cancer.org
American College of Gastroenterology	www.gi.org
American College of Physicians	www.acponline.org
American Gastroenterology Association	www.gastro.org
Centers for Disease Control	www.CDC.gov/vitalsigns
Colorectal Cancer Alliance	www.cca.org
Fight Colorectal Cancer	www.fightCRC.org
National Cancer Institute	www.cancer.gov/cancertopics/factsheet/detection/colorectal-screening
US Preventive Services Task Force	www.ahrq.gov/clinic/uspstfix.htm

To Place an Order or Find Out More

Please visit our website, EpigenomicsUSA.com, and select the “Q & A” tab where you will find many answers to commonly asked questions about the Epi proColon Test, the blood test for Colorectal Cancer Screening. To learn more about the Company and our products, or to place an order, please contact Customer Support at one of the following:

Customer Support	Customer Support@EpigenomicsUSA.com <placeholder>
US Toll Free	xxx.aaa.bbbb <placeholder>
Corporate	206.883.2900
Corporate FAX	206.254.9151

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